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A Study of the Morbid Changes which occur
in the Calcium Content of the Blood in
Rickets.

A Thesis submitted for the Degree of M.D.

By

A. G. Yates, M.A., M.B., Ch.B.,

House Physician, Sheffield Royal
Infirmary.



Introduction.

One of the most striking facts in the pathology of Rickets, is the diminution in the amount of mineral matter found in the bones. Healthy bone contains from 65 to 67 per cent, whereas rickety bone often contains only 25 to 35, and in some cases the percentage has been as low as 20.

This diminution is brought about almost entirely by a deficiency of calcium salts. A no less striking fact is that the bones are the only tissue concerned in this diminution, the amount of calcium in the soft tissues being as high or even higher than in health.

It is moreover well known that the particular types of diet which are most prone to produce rickets are in no way deficient in calcium, and, that the calcium deficiency produced by a calcium free diet in animals, is of a totally different type, affecting the soft tissues equally with the bones.

Rickets therefore is not primarily a calcium starvation, and it is reasonable to suppose that the calcium of the food, which for some reason has failed to find its way to the bones, will be found in the soft tissues, in the excretions, or in the blood. It has already been found in the soft tissues. The results of analyses of the faeces and urine are not constant. ^{*}Normal or subnormal excretion has been observed, /

*Metabolism and Practical Medicine by Von Noorden, Eng. Edition Vol. 3 P 1268.

"observed, others report an increase."

The calcium content of the blood in rickets has not so far as I am able to ascertain been determined during life, owing possibly to the elaborate and complicated methods hitherto employed. The method introduced some years ago by Sir A. E. Wright depending on the degree of concentration of a solution of Ammonium oxalate required to prevent the blood from coagulating, was a method of considerable clinical value, but he did not apply it to an investigation of the blood in this disease.

In the British Medical Journal of April 20, 1907, Dr Blair Bell of Liverpool published a method of determining the calcium content of the blood, which possessed the advantages of comparative simplicity and moderate amount of blood required. It has since been somewhat modified in certain of its details by the author.

The essential points in the determination as it is now carried out are, - 100 cubic millimetres of blood are withdrawn into a graduated pipette. This is thoroughly mixed in a glass capsule with a carefully measured quantity of oxalic acid solution 1 in 30, the calcium is thus precipitated as an oxalate.

The mixture is further diluted by adding a measured quantity of a solution containing acetic acid and glycerine in distilled water.

The/

The resultant fluid is examined on a Thoma Zeiss haemocytometer slide, and the crystals of oxalate counted.

The result is stated in terms of the number of crystals found per square, the unit being one. That is to say if on the 16 sets of 16 squares there are 256 crystals, the index of the calcium content is 1. By this method the calcium content of different individuals, and of the same individual at different periods can be rapidly and easily compared. * The author of this method showed that the calcium content of the blood presented certain variations according to the time of day at which the estimations were made. It was as a rule higher in the morning than at later periods of the day. The estimations contained in the present paper were made so far as was practicable at the same time of the day in order to avoid any error from this source.

Another possible source of error in carrying out determinations of this kind is a prejudice in the mind of the observer, derived from a knowledge of the kind of case from which the blood is taken. In order to avoid this, in many of the following cases, the capsules containing the blood were numbered by a second person, so that the case from which the blood was taken was not known by the observer until after the/

the count had been made. Professor Herbert Jackson of King's College had the method exhaustively tested in his laboratory and reported that it was an excellent clinical method.*1.

*2. T. Addis of Edinburgh also tested its accuracy and reported that the experimental error was 2.4 per cent.

In the following pages, I hope to record the results of an investigation into the changes found in the blood of rickety children, ^{by this method,} and to throw some light upon them by a series of observations in other cases where marked bony changes were taking place.

On the Normal Calcium Content.

Before presuming to say that the results obtained from the examination of the blood in cases of rickets show a definite morbid change, it is essential to know something of the normal calcium metabolism, and to have an idea approximately of what will be found in the healthy individual.

*3.

"In normal physiological conditions if the tissues are needing calcium, one would expect to find a low calcium content, that is to say the tissues will be taking up all the calcium they can get."

On/

*1. Brit. Med. Journal, Feb. 27, 1909, p. 518.

*2. Quarterly Med. Jour. Jan. 1909, Article by T. Addis on "The effect of the administration of Calcium salts and Citric Acid on the calcium content, and coagulation time of the blood."

*3. Brit. Med. Jour. April 27, 1909, p. 519. Article on calcium metabolism by Blair Bell & Pentland Hick.

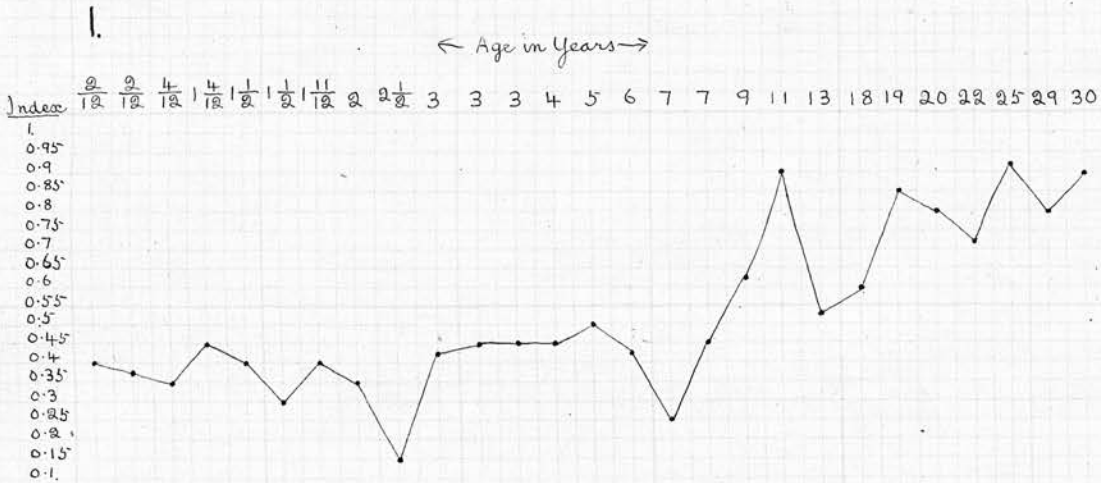
On the other hand when the tissues are taking up less calcium one would expect to find a higher index.

This is all the more likely to be the case as calcium salts are absorbed with considerable difficulty from the intestine, and the blood, therefore, cannot easily make up by absorption the deficiency caused by an increased deposit in the tissues.

During the period of growth in which the skeleton is becoming ossified and rapidly increasing in size, the demand for calcium must be considerably larger than at a later period, when the bones have attained full development. One would expect to find therefore, a low index during infancy and childhood becoming higher towards adult life.

The following chart represents a series of results obtained from healthy individuals, the vertical column representing the index obtained, and the horizontal line the ages of the individuals arranged in ascending order. It was necessary to select for this series, cases which were free not only from rickets, but also from any other condition which might presumably affect the calcium content of the blood. The cases chosen were cases of phimosis, hare lip, hernia and other similar surgical conditions, which could not affect the composition of the blood. The estimations were/

were made either prior to operation or during the period of convalescence.



It is seen from this chart that in infancy and early childhood the index obtained is a low one, being most frequently between 0.35 and 0.45. The two cases in which the lowest indices were obtained were particularly robust and vigorous children. Towards puberty and adult life the index rises considerably.

This series of observations is open to the objection that, as they were necessarily made from different individuals, other conditions which might influence the calcium content of the blood, may not have been equal.

In order to be sure that the calcium content is lower during the period of growth than in adult life, it/

it would be desirable to make a series of determinations on the same individual at different age periods. This is obviously impracticable in the human subject.

*

I have commenced a series of calcium estimations on growing rabbits in order to ascertain whether the calcium content increases as the animal grows larger, and the demand for lime for purposes of ossification becomes less.

Owing to the difficulty of applying the method to so small an animal as a young rabbit, I have so far only been able to get a consecutive series of 4 observations on one animal, and these were at rather wide and irregular intervals. So far as they go, however, they show the same thing.

Chart No. 2 shows these results:-

2	Date	Age in Weeks	Weight in Grams	Index
	March 1	4	252	0.41
	March 14	6	408	1.66
	March 22	7	438	1.45
	April 4	9	492	1.86

It will be seen that from the 4th to the 6th week/

*This work is being carried out in the Pathological Department of the University of Sheffield.

week the animal gained 156 grams, and that at the beginning of this period, the index was 0.41 whilst at the end of it, it had risen to 1.66.

From the 6th to the 9th week the gain was only 84 grams and the index remained high throughout this period.

These figures seem to show that while the growth and presumably also the ossification was rapid, the calcium content was low. When, however, the growth became less rapid the calcium content rose.

The next two charts afford additional evidence that a low calcium content is found when the tissues are taking up a large amount of lime from the blood.

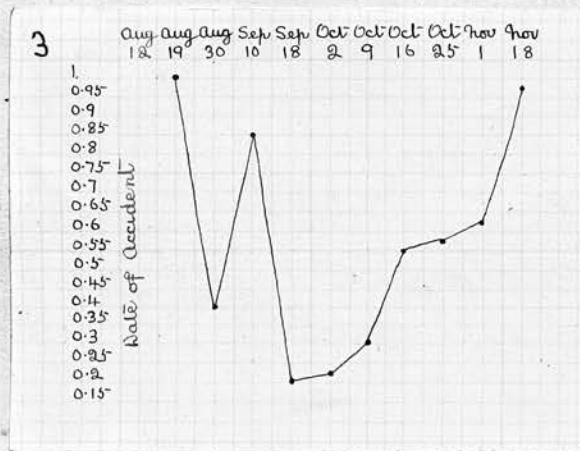


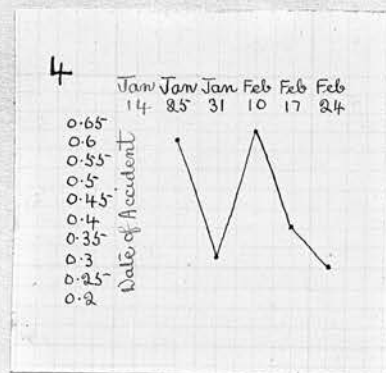
Chart 3 shows the results of periodical estimations of the calcium content in the case of a man 28 years of age who had sustained fractures of one leg, both forearms and the lower jaw.

In this case during the 4 weeks immediately following the accident with one exception, the index became/

became rapidly lower, it then began to rise steadily, and at the end of 11 weeks when the man had practically recovered, it was as high as it had been a week after the accident.

That is to say during the period in which a large quantity of calcium was required for the formation of callus and new bone, the calcium content became low, but when the process of consolidation was nearing completion, and callus was beginning to be absorbed, the index began to rise and when the process of healing was completed once more attained the normal level.

The case shown in chart 4 was unfortunately not followed through, but it shows the same thing as far as it goes.



The case was one of fractured femur in a man of 53. The initial fall is followed by a considerable rise during the 4th week which curiously corresponds with the rise seen in chart 3, which also occurred during the 4th week after the accident.

A third case of fractured femur in a man of 42 in which only two estimations were made showed a fall from 0.75 to 0.58 during the second week following the accident. To sum up briefly the above observations point to the fact that the calcium content of the blood is influenced to a marked degree by the demand of the tissues for calcium, being low when the demand is great, and vice versa, and further that in infants and young children the calcium content is a low one, the index being most frequently between 0.3 and 0.45.

On the Calcium Content in Rickets.

Most if not all pathological processes are disturbances of normal physiological action.

If the foregoing facts hold good for the healthy individual, in the rickety child one would expect to find the normal balance between the amount of calcium in the tissues and in the blood upset by the fact that the calcium is not being laid down in the bones in sufficient quantity. This disturbance of the normal relationship should take the form of an increase in the amount of calcium found in the blood. In carrying out a series of observations for the purpose of testing this, the first essential was to select ones cases carefully. It was necessary to be quite sure that at the time the determinations were made an active rickety process was going on.

The cases from which the following observations were made presented in addition to the usual skeletal changes, the constitutional symptoms of active rickets in a well marked degree.

The next chart shows the results obtained, in red, and for comparison, the results obtained from healthy children previously shown in chart 1, are inserted in black.



The most striking fact in this chart, is that up to the age of 9 without exception, the indices of the rickety children are higher than those obtained from healthy ones.

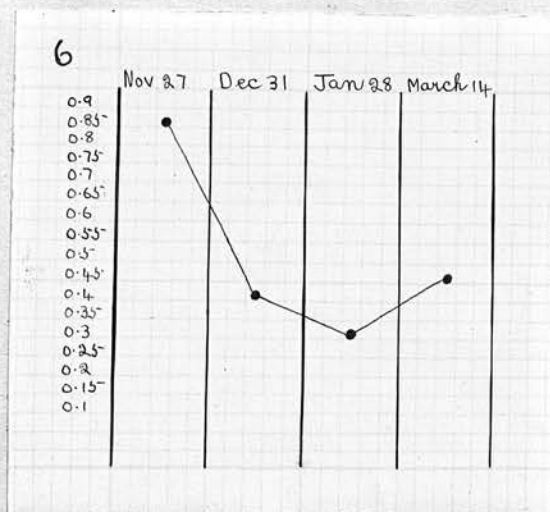
The lowest index obtained from a rickety child up to the age of 7 was 0.62 whereas during the same age/

age period the highest index obtained from a healthy one was 0.5.

Another fact which is seen on looking at this chart is the extreme irregularity of the results obtained from the rickety children. The indices vary from 0.62 to 1.5 and bear no relationship to the age of the patient.

A possible explanation of this is that rickets is a process which can vary in degree, and if the raised calcium content of the blood is a part of or depends upon the morbid changes that are going on, the more severe the rickets, the higher will the calcium content become. On examining the records of the cases, however, it was not found possible to estimate the severity of the condition with sufficient accuracy to be of any value in confirmation of this.

The following cases afford additional evidence that the calcium content of the blood in rickets is abnormally high. They demonstrate the effects of treatment.



Chart/

Chart 6 represents the case of a boy of 7, who though somewhat beyond the age at which rickets commonly occurs, at the time he came under observation, presented unmistakable signs of the disease.

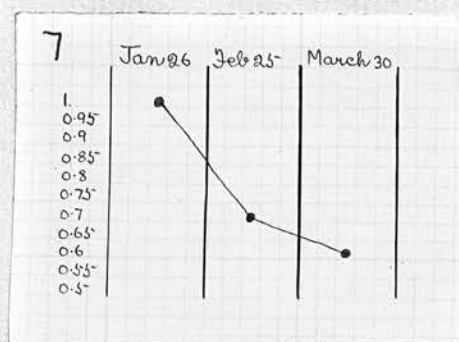
The actual occasion of his being brought to hospital was the increasing weakness of the legs, which caused him to fall down frequently. He had bronchitis, perspired freely about the head at night, and was very restless, he was extremely small and stunted for his age. The epiphyses were markedly enlarged at the wrists, ankles and costo-chondral junctions. The chest presented the characteristic deformities, and the legs were markedly bent. The liver was somewhat enlarged, and the abdomen distended. His calcium content when he first came to hospital on Nov. 27, was 0.89.

On Dec. 31. he showed very considerable improvement, he could walk perfectly well, the night sweating had ceased and his general appearance was much more satisfactory, his index was then 0.41. On Jan. 28 he had still further improved and his index was 0.33.

On March 14 he was apparently free from the signs of active rickets except, that some enlargement of the epiphyses remained, the index had risen to 0.45.

Chart 7 shows the same thing in a child of 2. After 2 months treatment the index was reduced from 1 to 0.62./

to 0.62.



In a third case in which only two estimations were made the index was 1.35 on July 31st and on Oct. 25 it had fallen to 0.68. In both cases the improvement was quite definite.

All these cases were treated as out-patients with the exception of the first who was an in-patient during the first month of the time. The treatment adopted was attention to hygienic surroundings and diet along with the administration of Cod Liver Oil, either alone or in combination with Extract of Malt. These observations though fewer in number than one might wish, show without exception that in active rickets a rise in the calcium content of the blood is a constant feature.

Any discussion of the cause of this, resolves itself into an endeavour to find out why the bones fail to take up calcium from the blood in sufficient quantity.

This failure must be due either to some fault in the/

the composition of the blood, or to some change in the osteoblastic cells, whose function is to take up lime and other materials from the blood and produce bone.

It is well known that calcium exists in the blood in 2 forms in one of which it is combined with protein, fatty acid or other organic material, in the other it is not so combined but occurs as an inorganic salt. These different combinations probably possess different properties especially as recent work on physical chemistry has shown that many substances and among them calcium salts may be either ionised or non ionised, the former being chemically the more active. This fact suggests the possibility of one of the organic forms being an intermediate stage in the process by which the calcium is taken up from the blood by the osteoblasts and deposited in the bones.

The possibility of some organic form of lime being essential to the process of ossification is supported by the fact that Pfaundler^{*} has shown that rachitic osteoid tissue is incapable of correcting its calcium deficiency by means of absorption from a solution of calcium chloride.

If this is the case the process must be somewhat as follows,- the calcium is absorbed from the alimentary tract and when it first gets into the blood occurs in/

*Diseases of Children. Shaw and La F  tra Vol. I, p. 14.

in the inorganic form, it then enters into some organic combination and in this form is taken up by the osteoblasts and used by them for the purpose of bone formation. Stoeltzner^{*} has differentiated two functions of calcium in relation to bone formation. He says that Calcium, -

1. Is itself laid down as bone.
2. Stimulates the osteoblasts to form bone.

The first function is of course obvious, the second he claims to have proved by experiments on animals.

If his theory is correct is it not possible that function No. 1 is performed by the organic calcium, whilst the stimulation of the osteoblasts is produced by the inorganic form?

We know from the work of Ringer and Locke that inorganic lime salts can stimulate heart and other forms of muscle, why should it not also exert a stimulant action on the osteoblastic cells.?

We may now further elaborate the process of calcium transference. It is taken up from the alimentary tract and gets into the blood in the inorganic form, a portion of this acts as a stimulant to the osteoblasts, whilst the other portion is transformed into an organic form and is taken up by these same osteoblasts and used by them for the purpose of bone/

* Archiv f. d. gesamte Physiologie Bd. CXXII p. 599
article by W. Stoeltzner.

bone formation. If this is the process in health, we must next inquire in what way it is disturbed in rickets. In this disease there is an excessive formation of osteoid tissue, but here the process stops short, and the deposition of lime by which the osteoid tissue becomes bone is deficient.

This is exactly what would occur if the osteoblasts were over stimulated, but were insufficiently supplied with calcium in the form in which they could take it up. They would by reason of the over stimulation produce osteoid tissue in excess, but owing to the insufficient supply of the necessary material, the osteoid tissue would remain soft and unossified.

Such excessive stimulation of the osteoblasts and deficient supply of assimilable lime is exactly the state of affairs which would be brought about, if for some reason, the inorganic calcium failed to be transformed into the organic form. Hence this deficiency of transformation is quite compatible with the known facts of the morbid anatomy of rachitic bone.

We have next to inquire whether it is also compatible with the ascertained facts concerning the blood.

Its direct effect upon the composition of the blood would be an excess of inorganic and a deficiency of organic calcium.

Chemists/

Chemists are not agreed as to whether oxalic acid is capable of precipitating all the calcium from the blood. Many hold that the inorganic form is alone precipitated or at any rate that some of the organic compounds are unaffected.

*

Sabbatine has shown that the calcium of the blood exists in two forms, a non-dissociable form in combination with protein molecules and a dissociable one. Oxalic Acid precipitates the latter, but the non-dissociable form is unaffected. C. J. Martin also has shown that Citric Acid diminishes the dissociable form by forming a citrate, which is further changed into a complex body, from which the calcium is not dissociable by Oxalic Acid.

If this is so, the results of the observations recorded in the earlier part of this paper apply only to the inorganic lime, and the increased calcium content found in cases of rickets represents the increase in the inorganic lime due to its deficient transformation into the organic form. The theory then that the underlying fact in the pathology of rickets is a deficient transformation of inorganic into organic compounds of calcium is compatible also with the state of the blood as revealed by investigations into its calcium content.

It is necessary to observe at this point that rickets is rightly regarded as a constitutional disease rather/

* Article on the Calcium Content and Coagulation time of the blood by T. Addis. Quarterly Med. Jour. Jan. 09.

rather than one of bone merely, but it is permissible to assume that a change in the composition of the blood so important as a considerable increase in the amount of its inorganic calcium compounds, would cause in addition to bony changes, others of a more general character, and our growing knowledge of the importance of calcium in the general metabolism of the whole body lends support to this view.

For example it has been shown experimentally that the irritability of a frog's nerve lasts much longer in a salt solution containing lime than in an unmixed salt solution. Hence lime acts as a stimulant to nervous tissue.

This fact suggests an explanation of the irritability, restlessness and by hyperaesthesia, which are such typical features of active rickets, in the long continued over stimulation of the nervous system produced by an excess of inorganic lime salts in the blood.

Finally it is necessary to ask whether it is possible to discover in the aetiological factors of rickets so far as we know them, an explanation of this change in the calcium content of the blood.

It is recognised on all hands that one of the most important causes of rickets if not the sole cause as Bland-Sutton suggests is a deficiency of fat in the food.

This/

This fact suggests that fat metabolism may be intimately associated with the process of bone formation.

The exact changes which fat undergoes after absorption are not fully known, but it is generally accepted that the first step in the process of oxidation is the splitting of the fat into a fatty acid and glycerine by the enzyme lipase contained in the tissues.

This cleavage is known to take place during the process of absorption of fat by the epithelial cells of the intestine. Moreover recent experiments have shown that a certain proportion of the absorbed fat does not reach the blood in the ordinary way via lacteals and thoracic duct, but is absorbed directly by the blood vessels of the intestine as fatty acids and soaps.

The group of substances included under the term organic calcium is probably a large one, and much has yet to be learnt concerning their exact nature, but in view of the fact that fatty acids are liberated within the body from fats after absorption, it is almost certain that they combine with calcium as with other alkalis and form calcium soaps.

*
Zweifel recently determined the amount of calcium salts in the faeces of rickety children and found that with/

with a greater quantity of fat, the calcium soap increased, and the calcium salts soluble in water diminished and vice versa. This shows that calcium salts readily form soap when fats are present in sufficient quantity.

Within the tissues also the existence of calcium compounds of the fatty acids has been abundantly proved. It is known to exist in some of the blood cells in combination with the fatty substance lecithin.

*
Dr Owen T. Williams has shown that calcium soaps can be found in small quantities in healthy intestinal mucous membrane by appropriate staining methods. It does not seem improbable therefore that some of the fatty compounds of calcium may be essential to the process of bone formation, and this would explain why fats are so important an element in the food during the period of growth.

If calcium in combination with a fatty acid is an intermediate stage in the process by which the inorganic lime salts of the blood are converted into the organic compounds which can be taken up by the osteoblasts, or if they are themselves the compounds which are so taken up, it is easy to realise how a diminished amount of fat in the food will bring^{about} this diminished transformation. The inorganic salts having insufficient fatty acid to combine with, cannot be converted in sufficient quantity into the organic compounds.

It/

* "The Aetiology of Appendicitis". Liverpool Med. Jour. July 1908.

It may be objected that a certain number of cases of rickets are fat and well nourished and that therefore there must be an abundance of available fat in the tissues.

If we look, however, at the character of the fats which possess in most marked degree anti-rachitic properties, we find that they are fluid at the ordinary temperature, easily absorbed and contain a high percentage of the fats which are glycerides of the unsaturated fatty acids. The fat of milk is nearly one half olein, moreover if we compare human milk with cows milk, we find that the former contains a higher total amount of fat and a larger proportion of olein, and that the fat of human milk has a higher Iodine value.

Raudnitz gives the following figures.- *

	<u>Human Milk</u>	<u>Cows' Milk</u>
Total Fat	5	3.4
Iodine value of fat	32 - 58	26 - 49
% of Olein	50	34

In other words, human milk contains a considerably higher proportion of unsaturated fat than cows' milk.

Cod Liver Oil has an Iodine value of 160 which is nearly 3 times that of ordinary fat, and must, therefore/

* Pfaunder & Schlossmann, Handbuch des Kinderheilkunde
Article "die Milch" by Raudnitz.

therefore contain a very large proportion of unsaturated fat. Heyderdahl has recently stated that it is composed entirely of these fats.

*1. Further, Freund has shown that the subcutaneous fat of breast fed children contains a higher percentage of unsaturated fat than that of artificially fed children as measured by the iodine absorption.

These facts seem to show that the fats which are glycerides of the unsaturated fatty acids are in some way essential to normal bone formation.

* 2. Dr Owen T. Williams in his study of the aetiology of appendicitis found that the calcium soaps of the unsaturated fatty acids were much more readily absorbed than the soaps of the saturated ones, and he showed that the former were produced in much greater abundance when the food contained a considerable proportion of unsaturated fat.

* 3. Freund again showed that in a rickety child the administration of Cod Liver Oil very markedly diminished the percentage of insoluble soaps in the faeces, and at the same time considerably increased the total amount of lime absorbed into the tissues.

These/

*1. Ergebnisse der Inneren Medizin und Kinderheilkunde
Bd. 3. 1909, p. 169.

*2. Liverpool Med. Jour. July 1908.

*3. Ergebnisse der Inneren Medizin und Kinderheilkunde
Bd. 3. 1909. p. 181.

These are his figures.-

Diet	Percentage of Insoluble soaps in the faeces.	Calcium Balance.
Carbohydrates with milk	57	+0.0313
Cod Liver Oil added	6.2	+0.2545

This also seems to show that the calcium compounds of the unsaturated fatty acids are more absorbable and probably also more soluble than the saturated compounds. Taking all these facts together the suggestion seems not improbable that the more absorbable and more soluble calcium soaps of the unsaturated fatty acids play an essential part in the chemical and physiological processes by which Calcium is taken from the blood and laid down as bone, and further, that the morbid changes characteristic of rickets are due to an insufficient formation of these unsaturated calcium soaps owing to a deficient supply of unsaturated fat in the food for the simpler compounds of calcium to combine with.

Summary.

In rickets the calcium content of the blood, as estimated by the oxalic acid method is considerably above the normal. The results obtained from healthy individuals and from cases of fracture show, that the calcium content is low when the tissues are using
up/

up a large quantity, and higher when the demand is less. Hence the raised content found in rickets is due to the fact that the bones are not taking up a sufficient quantity of lime from the blood.

The reasons given for this are purely theoretical but coincide with the facts so far as we know them.

They are briefly as follows.-

The calcium salts go through a series of chemical changes before they are ultimately laid down by the osteoblasts as bone.

The compounds of calcium with some of the unsaturated fatty acids are an essential link in this series of chemical changes.

In rickets there is a deficient supply of unsaturated fat in the food, and as a consequence these chemical changes cannot take place, and the calcium remains in the blood in the form in which it can be precipitated by the oxalic acid method. Hence clinically we find an increased calcium content.

In this discussion the question of the amount of calcium in the food has been practically ignored for the the following reasons. -

It is known that rickets is not due to a deficiency of calcium in the food, and it is unlikely that there was such a deficiency in any of the cases discussed.

On the other hand, by giving calcium salts by the/

the mouth you cannot raise the content of the blood much above the normal.

* Sir A. E. Wright showed that this was due to the fact that excessive dosage rendered the body immune or resistant to the absorption of calcium and especially active in its excretion.

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- * Liverpool Med. Journal, July 1908, Article on "The Physiological importance of the Calcium Salts". By W. Blair Bell.

